Biochemical Characterization of New P450s in Rice Diterpenoid Metabolism

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P450s

Background

- Involved in plant terpenoid Biosynthesis
- CYP76M subfamily
  - CYPs oxygenate products of diterpenes
  - Products act as fungicides and antibiotics
  - Similar amino acid sequences
  - Different specificities

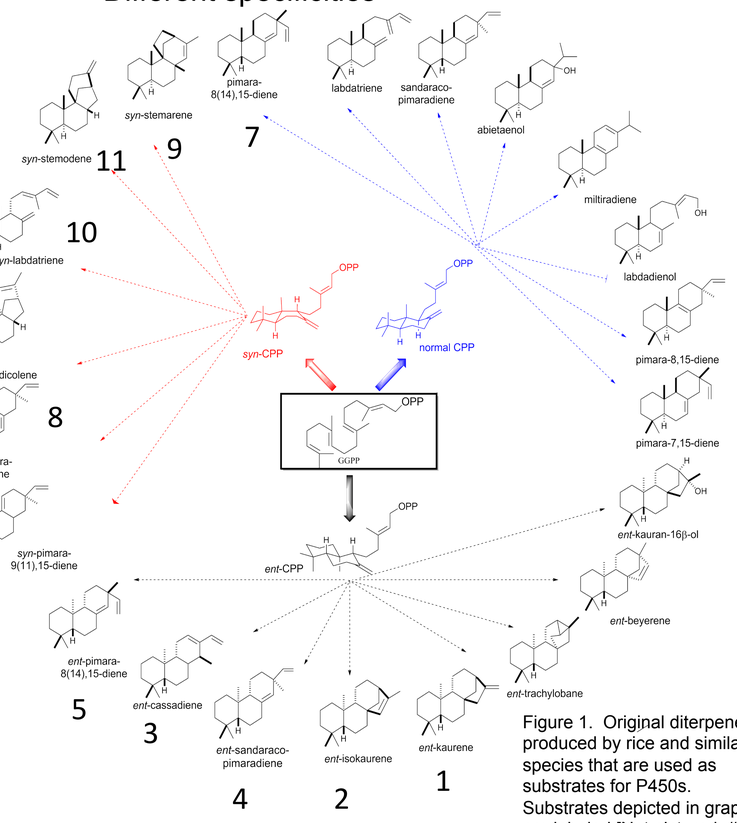


Figure 1. Original diterpenes produced by rice and similar species that are used as substrates for P450s. Substrates depicted in graphs are labeled [Not pictured, #6 iso-Pimara-8(14),15-diene].

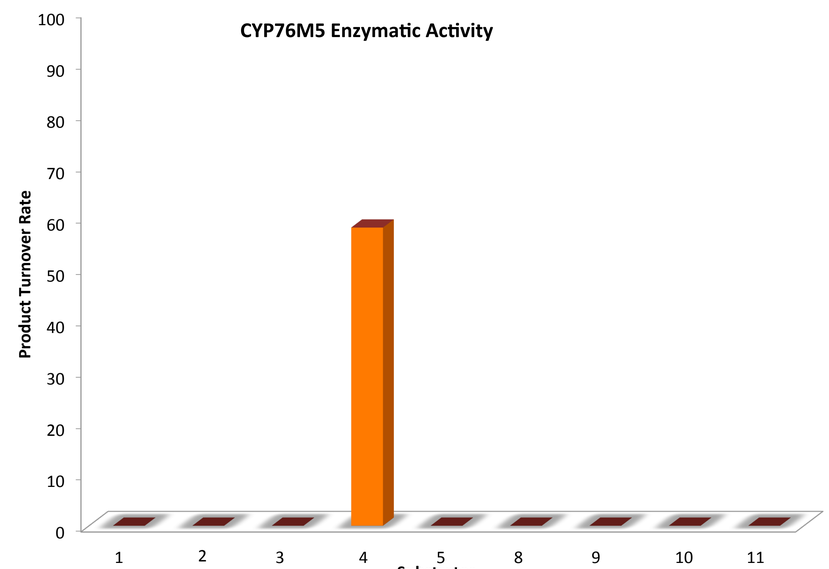
Previous Work

- Screened CYP76M5-8 against KSL products in *Escherichia coli*.
- Characterized majority of products.

Results

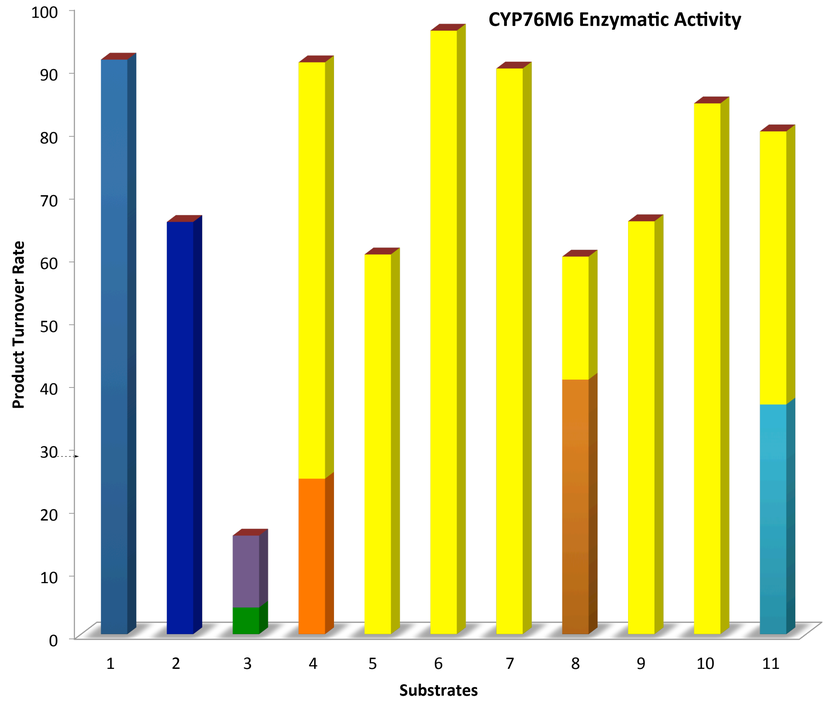
- 7α-hydroxy-ent-kaurene
- 7α-hydroxy-ent-isokaurene
- 7β-hydroxy-ent-sandaracopimaradiene
- 7β-OH-syn-stemodene
- 11α-hydroxy-ent-cassadiene
- 6β-hydroxy-syn-pimaradiene
- 6,12-hydroxysandaracopimaradiene
- 6β-hydroxy-syn-stemodene
- 7β-hydroxy-ent-pimaradiene
- 11-keto-cassadiene
- Oryzalexin E
- 11-hydroxy-cassadiene
- Unknown

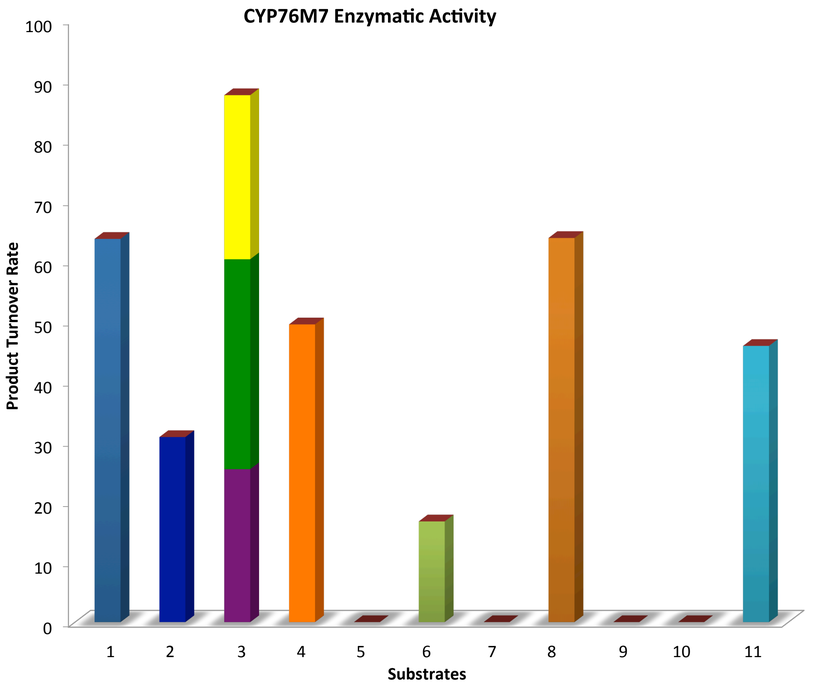
Previously Characterized P450s

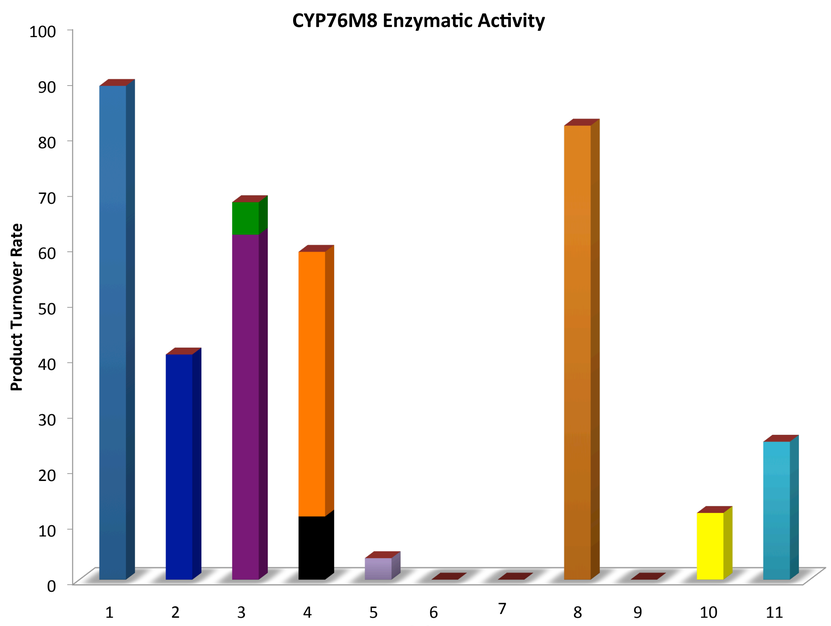


Results

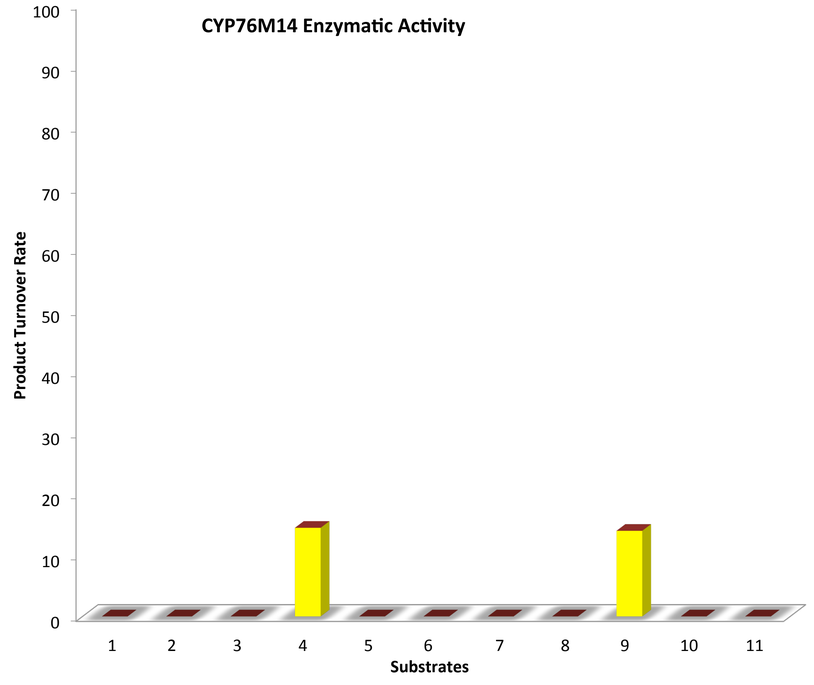
Previously Characterized P450s

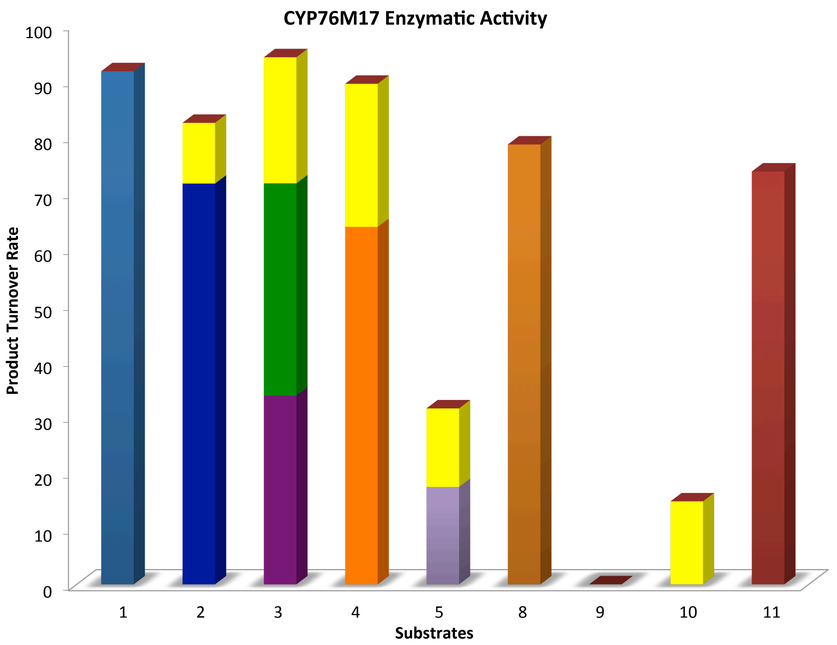






Newly Characterized P450s





Conclusion

- M17 is a more promiscuous enzyme similar to M8 while M14 has a high specificity similar to M5.
- M17 produces a novel product by hydroxylating the 7β position of syn-stemodene.

Future research

- Finish extracting unknown products from M14 & M17 to characterize.
- Perform feeding experiments on M14 to see if it works in tandem with another CYP76M enzyme.

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